

Effects-based monitoring in the lower Green Bay/Fox River and Milwaukee Estuary Areas of Concern using caged Fathead Minnows (*Pimephales promelas*).

Stevens, KE¹, Ankley, GT², Berninger, JP³, Cavallin, JE¹, Durhan, EJ², Jensen, KM², Kahl, MD², LaLone, CA², Makynen, EA², Severson, MN², Skolness, SY⁴, , Thomas, LM,⁵ Villeneuve, DL²,

¹ORISE Program, US EPA, Duluth, MN

²USEPA, Duluth, MN

³National Research Council, US EPA Duluth, MN

⁴University of Minnesota Duluth, US EPA Duluth, MN

⁵USFWS, Bloomington, MN

Abstract:

Within the Great Lakes there is an increased focus on contaminants of emerging concern (CECs) and their potential effects on aquatic organisms, including adverse reproductive effects. To further characterize the utility of caged fathead minnows (*Pimephales promelas*) for effects-based monitoring of CECs, we conducted in-situ exposures in the Lower Green Bay/Fox River, and Milwaukee estuary areas of concern (AOC). Sexually mature fathead minnows were exposed at multiple locations within the AOCs, particularly around industrial and waste water treatment plant (WWTP) discharges. Composite and grab water samples collected from each location were also analyzed for over 100 CECs and evaluated using in vitro bioassays (T47D-kbluc to evaluate estrogenic activity and MDA-kb2 to evaluate androgenic activity). No significant androgenic activity was detected with the MDA-kb2 assay. However, preliminary T47D results for both AOCs showed detectable estrogenic activity at roughly half the sites. Generally, this did not translate into significant hepatic vitellogenin mRNA induction in males caged at the same sites. However, expression of genes encoding hepatic xenobiotic metabolizing enzymes (e.g., *cyp1a1*, *cyp3a*) were significantly induced in male fathead minnows caged at some sites near WWTPs, compared to fish from the same cohort sampled on the day the fish were deployed (d 0 controls). Additionally, ex vivo estradiol and testosterone production by ovary tissue collected from females caged at sites near WWTPs in both AOC was significantly elevated. Initial analyses of targeted endpoints will be complemented with more open-ended transcriptomic and metabolomic analyses to further characterize biological responses at these locations.

Keywords: Endocrine disruption, Waste water treatment plant, Steroidogenesis, Great Lakes